SelectFX® Alexa Fluor® 488 Endoplasmic Reticulum Labeling Kit

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
S34253 SelectFX® Alexa Fluor® 488 Endoplasmic Reticulum Labeling Kit (S34200) 2-6°C Components				
Anti-protein disulfide isomerase (PDI), mouse IgG2b*	50 µL	500 µg/mL solution in PBS, 2 mM sodium azide	• 2–6˚C • Protect from light • DO NOT FREEZE	Components stable for up to 3 months.
Alexa Fluor® 488 goat anti–mouse IgG (H+L), highly cross-adsorbed*	50 µL	2 mg/mL solution in 0.1 M sodium phosphate, 0.1 NaCl, pH 7.5, 5 mM sodium azide		
Phosphate-buffered saline (PBS)*	100 mL	10X		
Blocking solution*	50 mL	10X, 100% heat-inactivated normal goat serum (NGS)		
S34252 SelectFX® Kits 2–25˚C Components				
Fixative solution	2 glass ampules (10 mL each)	4X, methanol-free 16% formaldehyde solution	• 2–25˚C • DO NOT FREEZE	Components stable for ~6 months.
Permeabilization solution	1.25 mL	100X, 20% solution of Triton X-100		
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* For long-term storage, any of these components may be divided into aliquots and stored at ≤–20˚C. Avoid repeated freezing and thawing of components stored at ≤–20˚C.

Approximate fluorescence excitation/emission maxima: ~495/519 nm for the Alexa Fluor® 488 dye conjugate. The labeling can be observed using standard fluorescein filter sets.

Introduction

The network of folded membranes that comprise's the endoplasmic reticulum (ER) in all eukaryotic cells constitutes greater than half of the total membrane in the average animal cell. The branching tubules and flattened sacs of the ER come in two forms: membrane bound by ribosomes (rough ER) or unbound by ribosomes (smooth ER). The ER has a central role in lipid and protein synthesis, protein chaperoning and folding, and calcium homeostasis. Abnormal protein folding in the ER is implicated in a number of diseases including cystic fibrosis and Alzheimer's disease.[1]

The SelectFX® Alexa Fluor® 488 Endoplasmic Reticulum Labeling Kit provides all the reagents needed to label the ER in fixed cells. To detect the ER, the kit uses a primary antibody directed against the ER-associated protein disulfide isomerase (PDI) and an Alexa Fluor® 488 dye–labeled secondary antibody; fluorescence is observed using standard fluorescein filters. The kit also includes cell fixative and permeabilization reagents and protocols for mammalian cell preparation and staining.

Before You Begin

Preparing Working Solutions

The working solutions can be prepared by mixing the entire contents of the supplied stock solutions at once, or on a per assay basis. Both methods are described below.

1.1 Prepare 1X PBS. To a one-liter container, mix 100 mL of 10X PBS and 900 mL of deionized water (dH_2O) to make a 1X PBS solution. For single assay preparation, add 1.0 mL of 10X PBS to 9.0 mL of dH_2O to make 10 mL of 1X PBS. Note that a portion of this solution will be used to prepare other working solutions, and the remainder will be used as a wash buffer. If the kit is being used for the first time, prepare an additional 30 mL of 1X PBS for use in making up the 1X fixative solution (see step 1.2). Store unused PBS at 2–6°C.

1.2 Prepare the 1X fixative solution. It is recommended that the entire contents of the ampule be used to make the working solution (preparing small amounts of fixative solution for each assay is not recommended). In a separate container, add the contents of one of the two supplied 10 mL ampules of 4X fixative solution to 30 mL of 1X PBS (prepared in step 1.1) to make a 4% fixative solution. The second ampule need not be opened until more 1X fixative solution needs to be made. Store unused 1X fixative solution at room temperature.

Note: The vial is designed to break at the narrow, scored neck. Exercise extreme care when opening the glass ampule of 4X fixative solution. First, hold the ampule vertically and tap it gently to ensure all of the fixative solution is in the body of the ampule. Then, using appropriate safety equipment to protect your hands and face, hold the ampule vertically and snap off the top.

1.3 Prepare the 1X permeabilization solution. In a separate glass container, mix 1.0 mL of the 100X permeabilization solution to 99 mL of 1X PBS (prepared in step 1.1) to make a 1X permeabilization solution of 0.2% Triton X-100. For single assay preparation, add 10 µL of the 100X permeabilization solution to 990 µL 1X PBS. Store unused permeabilization solution at room temperature.

1.4 Prepare the 1X blocking solution. In a separate container, mix 50 mL of the 10X blocking solution and 450 mL of 1X PBS (prepared in step 1.1) to make a 1X blocking solution consisting of 10% NGS. For single assay preparation, add 300 µL of 10X blocking solution to 2.7 mL of 1X PBS. Store unused blocking solution at 2–6°C.

Experimental Protocol

This protocol was developed using bovine pulmonary artery endothelial (BPAE) cells on coverslips but is broadly adaptable to other cell lines. Experimental parameters such as the amount of antibody used for staining and the incubation should be adjusted to achieve optimal staining. This protocol can also be adapted for use in conjunction with other probes for cellular targets for multicolor staining.

The protocol below, *ER Staining*, can be performed using adherent cells grown on a slide or coverslip. If nonadherent cells are used, deposit the washed cells onto a slide prior to staining.

Concentrated primary and secondary antibody solutions should be centrifuged at ~10,000 g for ~2 minutes at 4°C to sediment invisible aggregates before an aliquot is taken for the dilution (steps 2.7 and 2.9).

ER Staining

2.1 Wash the cells. Warm 1X PBS (prepared in step 1.1) to 37°C. Wash the cells once using 1.0 mL of warmed 1X PBS.

2.2 Fix the cells. Apply 0.8 mL of the 1X fixative solution (prepared in step 1.2) to the sample. Incubate for 15 minutes at 37°C.

2.3 Wash the cells. Wash the cells with 1.0 mL of room temperature 1X PBS. Repeat the wash.

2.4 Permeabilize the cells. Apply 1.0 mL of 1X permeabilization solution (prepared in step 1.3) to the sample. Incubate the sample at room temperature for 5 minutes.

2.5 Wash the cells. Wash the cells with 1.0 mL of room temperature 1X PBS. Repeat the wash.

2.6 Apply blocking solution. Apply 1.0 mL of 1X blocking solution (prepared in step 1.4) to the sample. Incubate the sample for 30–60 minutes at room temperature.

2.7 Apply the diluted primary antibody solution to the sample. Prepare a 1,000-fold dilution of the anti-PDI antibody by centrifuging the tube containing the anti-PDI antibody and adding 1.0 µL of the antibody solution to 1.0 mL of 1X blocking solution (prepared in step 1.4). Mix well, add the diluted antibody solution to the sample, and incubate at room temperature for 1–2 hours.

2.8 Wash the cells with 1.0 mL of 1X blocking solution. Repeat the wash 3–4 times.

2.9 Apply the diluted secondary antibody solution to the sample. Prepare a 1,000-fold dilution of the Alexa Fluor® 488–labeled secondary antibody by centrifuging the tube containing the secondary antibody and adding 1.0 µL of the antibody solution to 1.0 mL of 1X PBS. Mix well, add the diluted secondary antibody staining solution to the sample, and incubate at room temperature for 30 minutes protected from light.

2.10 Wash the cells with 1.0 mL of 1X PBS. Repeat the wash 3–4 times.

2.11 If desired, counterstain the cells with DAPI or other nucleic acid stain.

2.12 Mount the cells. For best results use an antifade reagent such as ProLong® Gold antifade reagent. View the sample with a fluorescence microscope equipped with filters appropriate for fluorescein.

Reference

1. Methods Mol Biol 232, 231-43 (2003).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
A11029	Alexa Fluor® 488 goat anti-mouse IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
P36930	ProLong® Gold antifade reagent	10 mL
P36931	ProLong® Gold antifade reagent with DAPI	10 mL
P36934	ProLong® Gold antifade reagent *special packaging*	5 x 2 mL
P36935	ProLong® Gold antifade reagent with DAPI *special packaging*	5 x 2 mL
S34200	SelectFX® Alexa Fluor® 488 Endoplasmic Reticulum Labeling Kit *for fixed cells*	1 kit

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